AD_____

Award Number: DAMD17-00-1-0637

TITLE: The RhoC Transgenic Mouse as a Realistic Model of Inflammatory Breast Cancer

PRINCIPAL INVESTIGATOR: Kenneth L. Van Golen, Ph.D.

CONTRACTING ORGANIZATION: The University of Michigan
Ann Arbor, Michigan 48109-1274

REPORT DATE: October 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2002	Final (15 Sep 00 - 14 Sep 02)

4. TITLE AND SUBTITLE
The RhoC Transgenic Mouse as a Realistic Model of Inflammatory Breast Cancer

5. FUNDING NUMBERS
DAMD17-00-1-0637

6. AUTHOR(S):
Kenneth L. Van Golen, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Michigan
Ann Arbor, Michigan 48109-1274

E*Mail: kgolen@umich.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

none provided

14. SUBJECT TERMS
breast cancer

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Final Report for DAMD17-00-1-0637

The RhoC Transgenic Mouse as a Realistic Model of Inflammatory Breast Cancer.

Principal Investigator, van Golen, Kenneth L., Ph.D.
Current address:
200 Zina Pitcher Place
R-4048 Kresge II
The University of Michigan Medical Center
Ann Arbor, MI 48109-0548

Phone: 734-615-6887
Fax: 734-615-2719
Email: kgolen@umich.edu

Introduction

Inflammatory breast cancer is a unique and highly aggressive form of locally advanced breast cancer[1,2]. Although it effects a small proportion of women with breast cancer annually in the United States (approximately 6%), it carries with it the worst prognosis of all breast cancers[1,3]. Inflammatory breast cancer is unique in the aspect that it invades and grows within the dermal lymphatics of the breast. Blockage of the dermal lymphatics by tumor emboli leads to edema and swelling of the breast, thus giving the appearance of inflammation[1,2,3]. The tumor emboli can also metastasize via the dermal lymphatics to the skin of the contralateral breast, chest and stomach.

Although the clinical characteristics of inflammatory breast cancer were well documented, nothing was known about the genetic or molecular mechanisms involved in conferring the unique phenotype to this disease. In a comprehensive study aimed at discovering the genetic mechanisms underlying inflammatory breast cancer growth and metastasis, our laboratory found that RhoC GTPase was overexpressed in over 90% of inflammatory breast cancer patient samples analyzed (compared with 38% of stage-matched non-inflammatory tumors)[4]. RhoC GTPase is an oncogene which belongs to the Ras superfamily of small GTP binding proteins and is implicated in cellular motility and invasion. Subsequent experiments have demonstrated that overexpression of RhoC in normal human mammary epithelial cells can nearly recapitulate the inflammatory breast cancer phenotype[5,6].

No true model of inflammatory breast cancer development currently exists. Two alternative models; orthotopic injection of inflammatory breast cancer cell lines (the SUM149 and SUM190) into the mammary fat pad of nude mice, and the MARY-X xenograft, are the only ways of study inflammatory breast cancer growth[5,7]. Although, these models are useful for various aspects of inflammatory breast cancer research, they both utilize cells that were isolated after chemotherapeutic intervention. In order to better address inflammatory breast cancer development and growth we proposed to develop a RhoC transgenic mouse, modeled after the Her2/neu transgenic mouse[8]. We therefore attempted to clone RhoC into a tetracycline (tet) inducible vector containing a mouse mammary tumor virus (MMTV) promoter and develop RhoC transgenic mice within the one year concept award period.

Report Body

As outlined in the proposal, we first would attempt to produce a tet-inducible, MMTV-RhoC expression vector, transfect it into immortalized mammary epithelial cells, determine basal expression and tet-induced expression. Next we would develop a RhoC transgene screening method to screen the microinjected embryonic stem cells and eventually RhoC transgenic mice.

Unfortunately we had some unforeseen experimental problems early on in the project. The first problem was obtaining a tetracycline inducible MMTV-vector. At the time the proposal was written this vector was commercially available from Clonetech. After the funding period began, the vector (and the corresponding control vectors) had been discontinued and were no longer available. Several investigators were contacted and a vector was finally obtained. The vector had to be modified by us to contain restriction sites within the cloning cassette that would accept the RhoC gene. While in the process of obtaining and modifying the vector, we also obtained the pSWITCH system from Invitrogen. This system is a CMV-promoter system which uses a steroid type drug, mifepristone, to shut off the control vector and induce transgene transcription. Utilization of this CMV-promoter system would result in whole-body expression of RhoC, which would be extremely interesting in that no knowledge exists as to how this gene effects development, growth, etc. Unfortunately, the mifepristone system produced high basal expression in the human mammary epithelial cells, presumably due to the hormone

responsiveness of these cells. So, a conventional tet-inducible system was utilized similar to the MMTV-promoter system.

The next problem that occurred was the production of stable-tet-inducible MMTV-RhoC (and CMV-RhoC) human mammary epithelial cells. Although in the past we have had high transfection efficiencies with other expression vectors, utilizing the FuGene6 transfection reagent from Roche Biochemicals, transfection efficiency was very low and selection resulted in no viable clones. The transfection protocol was eventually optimized and stable tet-inducible clones were produced. Induced levels of RhoC were analyzed and found to be ~10-fold over basal, non-induced levels (approximately the level seen in human tumors). These tet-inducible cells will provide a valuable reagent for future *in vitro* studies.

Southern blot and PCR screening for the RhoC transgene have been performed and optimized. In addition a PCR titration curve of MMTV-RhoC (or CMV-RhoC) diluted in mouse tail DNA has been produced. This is a significant step in order to be able to detect 1 copy of the transgene in the resulting transgenic mice. Subsequently, the prokaryotic vector sequences of the tet-repressor and RhoC containing vectors have been trimmed away and the transgene has been injected into mouse embryonic stem cells.

The selection process of the embryonic stem cells has been completed. We have screened 192 stem cell clones for expression of the tet-repressor and RhoC transgene. Ten stem cell clones for each MMTV- and CMV-RhoC constructs have been selected and implanted into recipient mice. Over 500 mouse pups have been screened for transgene expression. The frequency of the RhoC transgene in the pups has been quite low, less than 20% of the mice carry the gene. However, these mice are currently being "back-crossed" with C57 black mice to provide a pure, stable genetic background containing the transgene.

We are now on the F2 generation and need to continue the back-cross until the F6 generation. When the F6 generation is reached, we will bank the mice with either the NIH or Jackson labs mouse repository. Also, at that time our experiments will begin with the F6 generation.

Key Research Accomplishments

- Development of tetracycline inducible MMTV-RhoC and CMV-RhoC expression vectors.
- Development of normal human mammary epithelial cell lines which contain the tetracycline inducible MMTV-RhoC and CMV-RhoC expression vectors. These cell lines will prove invaluable for future *in vitro* studies.
- Optimization of transgene screening methods for determining the presence of the RhoC gene in tissue culture cells, embryonic stem cells and transgenic mice.
- Successful introduction of the tetracycline inducible MMTV-RhoC and CMV-RhoC expression vectors into mouse embryonic stem cells.
- The mice that contain the RhoC-transgenes are viable and fertile.

Reportable Outcomes

It is premature to report any of the outcomes thus far. Progress has been made in overcoming technical obstructs that will allow for rapid progress to be made.

Conclusions

Development of the RhoC transgenic mouse has proven more difficult than originally anticipated. The major stumbling blocks lay in the development of the expression vector. One of the positive aspects of the initial experimental problems has been that in addition to the MMTV-RhoC vector, we were able to develop a CMV-RhoC vector. Basically, we are able to get "2 for the price of 1". Each will answer significant questions in the development of inflammatory breast cancer development. When the RhoC transgenic mouse is complete, we will be able to induce expression with tetracycline at the time of our choosing and in the case of the MMTV-RhoC mice have specific mammary targeted expression. In the case of the CMV-RhoC mice we will have whole body expression. This will answer questions of RhoC biology that remain a mystery such as its role in development.

References

1. Kleer, C. G., van Golen, K. L., and Merajver, S. D. Molecular biology of breast cancer metastasis: Inflammatory Breast Cancer: Clinical Syndrome and Molecular Determinants. Breast Cancer Research, *2*: 423-429, 2000.
2. Beahrs, O., Henson, D., and Hutter, R. Manual for Staging of Cancer., pp. 145-150. 1988.
3. Jaiyesimi, I., Buzdar, A., and Hortobagyi, G. Inflammatory Breast Cancer: A Review. J Clin Oncol, *10*: 1014-1024, 1992.
4. van Golen, K. L., Davies, S., Wu, Z. F., Wang, Y., Bucana, C. D., Root, H., Chandrasekharappa, S., Strawderman, M., Ethier, S. P., and Merajver, S. D. A novel putative low-affinity insulin-like growth factor-binding protein, LIBC (lost in inflammatory breast cancer), and RhoC GTPase correlate with the inflammatory breast cancer phenotype. Clin Cancer Res, *5*: 2511-2519, 1999.
5. van Golen, K. L., Wu, Z. F., Qiao, X. T., Bao, L. W., and Merajver, S. D. RhoC GTPase, a novel transforming oncogene for human mammary epithelial cells that partially recapitulates the inflammatory breast cancer phenotype. Cancer Res, *60*: 5832-5838, 2000.
6. van Golen, K. L., Wu, Z. F., Qiao, X. T., Bao, L. W., and Merajver, S. D. RhoC GTPase Overexpression Modulates Induction of Angiogenic Factors in Breast Cells. Neoplasia, *2*: 418-425, 2000.
7. Alpaugh ML, Tomlinson JS, Shao ZM, Barsky SH. A novel human xenograft model of inflammatory breast cancer. Cancer Res. 59(20):5079-84, 1999.
8. Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD, Muller WJ. Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. Proc Natl Acad Sci U S A 89(22):10578-82, 1992.